Journal of Experimental Marine Biology and Ecology 381 (2009) S173–S179

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Hypoxia tolerance of juvenile weakfish (*Cynoscion regalis*): Laboratory assessment of growth and behavioral avoidance responses

Kevin L. Stierhoff *[,1], Robin M. Tyler [2], Timothy E. Targett

University of Delaware, College of Marine and Earth Studies, Lewes, DE 19958, USA

ARTICLE INFO

Keywords:
Avoidance behavior
Growth
Hypoxia
Nursery habitat
Weakfish

ABSTRACT

Laboratory experiments were conducted to examine the growth rate and avoidance behavior of juvenile weakfish (*Cynoscion regalis*) exposed to hypoxia (dissolved oxygen (DO) below saturation). Growth rates were measured under constant (2.0, 3.5, 5.0 and 7.0 mg O_2 L^{-1}) and diel-cycling (2.0–11.0 mg O_2 L^{-1}) DO conditions at three temperatures (20, 25, and 30 °C) over 7 days. Two-way DO choice trials were conducted over the same temperature range to 1) determine the DO level at which juvenile weakfish exhibit hypoxia avoidance and 2) examine whether temperature influences hypoxia avoidance behavior. There was no significant effect of constant hypoxia, or diel-cycling hypoxia, on growth rates of weakfish at any temperature. In the DO choice trials, weakfish avoided 1 mg O_2 L^{-1} but showed no preference for DO when the low choice was ≥ 2 mg O_2 L^{-1}. Temperature had no significant effect on growth or avoidance responses of juvenile weakfish. Lack of mortality during seven days exposure to hypoxia as low as 2 mg O_2 L^{-1}, moderate to high growth rates, and lack of hypoxia avoidance of DO concentrations as low as 2 mg O_2 L^{-1} indicates that juvenile weakfish are physiologically capable of tolerating levels of hypoxia commonly encountered in estuarine nurseries during summer months. There was no indication that hypoxia avoidance occurs at a DO level higher than that which negatively impacts growth rate in juvenile weakfish.

Published by Elsevier B.V.

1. Introduction

Hypoxia, or dissolved oxygen (DO) below saturation, is considered a major threat to the value of estuarine habitats as nurseries for ecologically and economically important fishes (Kennish, 2002). The functional value of estuarine habitats as nurseries for young fishes is attributed primarily to physicochemical regimes (e.g., temperature and salinity) that are physiologically suitable or optimal, abundant prey resources, and low predation risk, all of which promote rapid growth and enhance survival (Joseph, 1973; Miller et al., 1985; Gibson, 1994). Since size-selective mortality processes such as predation and over-winter survival during larval and juvenile stages are thought to play a major role in regulating recruitment (Anderson, 1988; Sogard, 1997), negative effects of sublethal hypoxia on growth rate may decrease the value of certain estuarine areas as nurseries by decreasing survival.

Estuarine habitats along the east coast of the U.S. commonly experience summertime hypoxia. Constant severe hypoxia (≤ 2 mg O_2 L^{-1}) and anoxia (<0.2 mg O_2 L^{-1}) occur seasonally in deep areas of the Chesapeake Bay (Officer et al., 1984), Long Island Sound (Parker and O'Reilly, 1991; Anderson and Taylor, 2001), and Pamlico Sound, NC (Paerl et al., 1998). In contrast, severe diel-cycling hypoxia has been observed for extended periods of time in shallow coastal lagoon systems such as Waquoit Bay, MA (D'Avanzo and Kremer, 1994) and Delaware's Coastal Bays (Tyler et al., 2009). In his review, Diaz (2001) illustrates that the incidence and severity of hypoxia in coastal waters is increasing worldwide. This situation underscores the importance of understanding the effects of low DO on the physiological and behavioral ecology of estuary-dependent fishes, species that utilize estuaries as nurseries during their larval and/or juvenile stages.

Hypoxia-induced reductions in growth rate have been observed in juveniles of several estuary-dependent species, including spot (*Leiostomus xanthurus*, McNatt and Rice, 2004), southern flounder (*Paralichthys lethostigma*, Taylor and Miller, 2001), black sea bass (*Centropristis striata*, Hales and Able, 1995), winter flounder (*Pseudopleuronectes americanus*, Bejda et al., 1992; Stierhoff et al., 2006) and summer flounder (*Paralichthys dentatus*, Stierhoff et al., 2006). Among these species, significant reductions in growth rate occurred over a wide range of DO conditions. The severity of hypoxia necessary to suppress growth has often been shown to be temperature-dependent, with less severe hypoxia causing greater growth detriment at higher temperatures. Hypoxia levels necessary to cause reduced growth rates in the above species ranged from levels as high as 5 mg O_2 L^{-1} in some species to 1.5 mg O_2 L^{-1} in others.

Less information is available on the behavioral avoidance of hypoxia by estuary-dependent fishes. Such information is important

---

* Corresponding author: Tel.: +1 858 546 7180; fax: +1 858 546 5651.
E-mail address: kevin.stierhoff@noaa.gov (K.L. Stierhoff).

[1] Present address: NOAA Fisheries, Southwest Fisheries Science Center, 8604 La Jolla Shores Drive, La Jolla, CA 92037, USA.

[2] Present address: Delaware Department of Natural Resources and Environmental Control, Division of Water Resources, 89 Kings Highway, Dover, DE 19901, USA.

0022-0981/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.jembe.2009.07.020

to understanding the potential impacts of hypoxia, since hypoxia effects on fish growth are related to the ability of a particular species to detect and avoid potentially harmful environments. For example, juvenile spot in binary choice experiments only avoided the lowest DO treatment level (1 mg O_2 L^{-1}, Wannamaker and Rice, 2000), a DO level that is near lethal for this species (12 h median lethal concentration (LC_{50}) at 30 °C of 1.10 mg O_2 L^{-1}, Shimps et al., 2005) and is below that which resulted in reduced growth in the laboratory (1.5 mg O_2 L^{-1}, McNatt and Rice, 2004). Thus, this species would not be expected to vacate hypoxic areas in the field until after growth limitation has already occurred.

Information is needed on the relative effects of hypoxia on growth and avoidance for other estuary-dependent species to better assess the physiological and ecological consequences of hypoxia for estuary-dependent fishes. The weakfish, *Cynoscion regalis* (family Sciaenidae), is an ecologically and economically important estuary-dependent species distributed from Nova Scotia to Cape Canaveral, FL (Able and Fahay, 1998). Weakfish larvae and juveniles inhabit most Middle Atlantic Bight estuaries during summer nursery periods (Able and Fahay, 1998). Juveniles make extensive use of open-water estuarine habitats (e.g., Delaware Bay, Lankford and Targett, 1994; Grecay and Targett, 1996; Paperno et al., 2000) as well as shallow tidal tributaries (Litvin and Weinstein, 2003; Tyler and Targett, 2007) where they commonly experience some degree of hypoxia. To our knowledge there is no information on the effects of DO on growth, survival or behavior of larval, juvenile, or adult weakfish.

This study examined the effects of hypoxia on growth rate and avoidance behavior of juvenile weakfish in the laboratory. Growth rates were measured during exposure to constant and diel-cycling DO over a range of temperatures and under *ad libitum* feeding conditions. Two-way DO choice trials were conducted at the same temperatures to 1) determine the DO level at which juvenile weakfish exhibit hypoxia avoidance and 2) examine whether temperature influences hypoxia avoidance behavior.

2. Materials and methods

2.1. Collection and holding of experimental fish

Juvenile weakfish were collected from Pepper Creek (near Lewes, Delaware, USA) between June and August of 2002 and 2003. Fish were acclimated in the laboratory to treatment temperatures (≥14 d) and 20‰ salinity (≥7 d) at saturated DO conditions prior to experiments. Fish were held at 14 h light:10 h dark photoperiod and were fed frozen mysid shrimp (*Mysis relicta*) *ad libitum* twice daily.

2.2. Growth experiments

2.2.1. Experimental procedures

Juvenile weakfish were exposed to five DO treatments: normoxia (7.0 mg O_2 L^{-1}), constant hypoxia (2.0, 3.5, and 5.0 mg O_2 L^{-1}), and diel-cycling hypoxia (2.0–11.0 mg O_2 L^{-1}). Three temperatures (20, 25, and 30 °C) were tested in successive experiments. Salinity was maintained at 20‰ for all experiments. Minimum (2.0 mg O_2 L^{-1}) and maximum (11.0 mg O_2 L^{-1}) DO concentrations in the diel-cycling DO treatment were chosen to reflect those commonly occurring in eutrophied estuarine systems during the summer nursery period (Tyler et al., 2009), and coincided with the beginning of the light (0700 h) and dark (2100 h) periods, respectively. The lowest DO treatment (2.0 mg O_2 L^{-1}) was chosen to approach the lethal concentration for weakfish, based on reported values for other estuary-dependent fishes (Poucher and Coiro, 1997; Miller et al., 2002).

Dissolved oxygen was regulated using a computer-controlled device described in detail by Grecay and Stierhoff (2002). This device simultaneously regulated constant or diel-cycling DO in each of five 500-L recirculating aquarium systems. Each aquarium system had ten 18-L tanks with one fish in each. The DO level in each system was measured every 10 min and if adjustment was necessary DO was increased or decreased by the addition of either compressed O_2 or N_2 gas to the head reservoir. DO concentrations were maintained near treatment levels throughout the experiments (Table 1). Dissolved oxygen concentrations in each system were verified twice daily using a Yellow Springs Instruments (YSI) Model 55 handheld DO meter.

Juvenile weakfish (41–75 mm standard length, SL) were acclimated in the five aquarium systems (1 fish per tank; 10 fish per system) for 72 h before the beginning of experiments. Dissolved oxygen in each system was decreased to treatment concentrations during the final 24 h of acclimation. Fish were exposed to DO treatments for 7 d and fed *ad libitum* on frozen *M. relicta* twice daily (0900 h and 1700 h). Food was always available, except for 15 h prior to initial and final weighing to minimize the effect of stomach content mass on growth rate estimates. Uneaten food was removed daily before the 0900 h feeding to maintain food and water quality.

Specific growth rate (% mass d^{-1}) was calculated from the instantaneous growth rate (G) as: $SGR=(e^G-1)*100\%$; where $G=(\ln M_f-\ln M_i)*d^{-1}$, M_f = final wet mass, M_i = initial wet mass, and $d=7$ day duration of the experiment (Ricker, 1975). Linear growth rate (mm d^{-1}) was calculated as: $LGR=(L_f-L_i)*d^{-1}$; where L_f = final SL, L_i = initial SL, and $d=7$ day duration of the experiment.

2.2.2. Statistical analyses

The effect of DO and temperature on SGR and LGR was determined using a two-way analysis of covariance (ANCOVA) ($\alpha=0.05$) using initial fish mass as a covariate. Data used in all analyses were normally distributed (determined by one-sample Kolmogorov–Smirnov tests using the Lilliefors option). SGR data were logarithmically ($\log_{10}$) transformed as necessary to reduce heteroscedasticity. Statistical tests were conducted using SPSS software (SPSS, 2003).

2.3. Behavioral avoidance experiments

2.3.1. Experimental procedures

Two-way DO choice trials were conducted in replicate tanks with dimensions of 1 m (W)×2 m (L)×0.3 m (D) and volume of ~600-L. Trials were run at 20, 25, and 30 °C and a salinity of 20‰. The length of each tank was divided in half by a clear acrylic partition, which allowed the establishment of different DO concentrations on either side. Tanks were surrounded by black plastic curtains to minimize the

Table 1
Temperature, nominal dissolved oxygen (DO) treatments, and actual DO concentrations in juvenile weakfish (*Cynoscion regalis*) growth experiments.

T (°C)	DO treatment (mg O_2 L^{-1})	Mean ± (SD) DO (mg O_2 L^{-1})
20	2.0	2.20 (0.90)
	3.5	3.68 (0.84)
	5.0	5.09 (0.97)
	7.0	7.14 (1.23)
	Diel (min) 2.0	2.55 (1.62)
	Diel (max) 11.0	10.75 (1.75)
25	2.0	2.02 (0.78)
	3.5	3.41 (0.78)
	5.0	4.86 (0.78)
	7.0	6.78 (1.03)
	Diel (min) 2.0	2.37 (0.91)
	Diel (max) 11.0	10.43 (2.00)
30	2.0	2.24 (0.52)
	3.5	3.54 (0.91)
	5.0	5.14 (2.66)
	7.0	7.04 (1.30)
	Diel (min) 2.0	2.52 (1.04)
	Diel (max) 11.0	10.36 (2.72)

Mean DO in each constant treatment was calculated from triplicate measurements taken twice daily in each system using a handheld DO meter. Mean DO in each diel-cycling treatment was calculated from triplicate DO measurements taken at 0700 h (minimum DO) and 2100 h (maximum DO).